SENSITIZATION TO "TETRYL."

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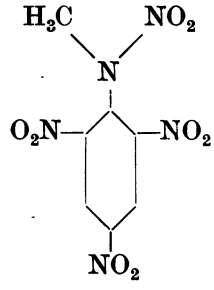
Received for publication September 1, 1944.

DURING the present war there has been a high incidence of contact dermatitis among workers in ordnance factories, particularly among those engaged in handling the explosives "tetryl" (trinitrophenylmethylnitramine) and fulminate of mercury. The present investigation of the mechanism of skin sensitization to these substances was undertaken in the hope of devising a method for diminishing the considerable loss of labour-time involved. This paper describes experiments with "tetryl" which, although they have not led to a solution of the practical problem of protection against sensitization, have revealed some points of general interest in relation to skin sensitivity. With fulminate no more has been done so far than to show that it is a powerful sensitizing agent for guinea-pigs when given by the intradermal route.

"Tetryl" falls into the class of trinitrobenzene explosives—



"Tetryl,"

which includes picric acid (lyddite) and trinitrotoluene (TNT). Both these are liable to sensitize the skins of those exposed to them, but do not do so nearly as frequently as does "tetryl." "Tetryl" presents in fact a contrast to TNT in that its systemic toxicity is inconsiderable (Cripps, 1917; Wells, Lewis, Sansum, McClure and Lussky, 1920; Noro, 1941; Horner, 1942; Witkowski, Fischer and Murdock, 1942) and its sensitizing potency high, whilst the reverse is true of TNT. It is not a direct irritant to the skin, although it is very irritating to the eyes, probably for physical reasons. Workers in the factories are exposed to direct skin-contact while handling the substance, and to inhalation of the dust; in spite of all precautions the skins of these workers are almost always stained, and the proportion of workers not previously exposed who become sensitized may be as high as 30 per cent. The "incubation period" for sensitization is about 10–14 days. Preventive measures aim at minimizing contact by reducing direct handling as far as possible, by diminishing dust in the shops by proper ventilation, and by the use of non-fatty barrier creams; and workers are instructed to remove all traces after work by washing with a 1 per cent. sulphite soap which decomposes "tetryl," giving a bright red colour. The only reliable method is to

take permanently out of contact all workers who show any signs of sensitivity, and only to employ those who are found by experience to be resistant. This is the course usually followed in peace-time, when the shortage of labour is not a factor. (Schwartz, 1944.)

The present investigation describes experimental methods of sensitizing guinea-pigs to " tetryl," together with a study of the chemical grouping in the molecule of " tetryl " which is responsible for its sensitizing properties, and some experiments bearing on the relation of skin sensitivity to other immune reactions.

EXPERIMENTAL.

Methods of Sensitization of Guinea-pigs.

Five methods of sensitization were used : (1) Intradermal injection ; (2) application to a scalded area ; (3) subcutaneous implantation of capsules ; (4) inunction in a lanoline base ; (5) inhalation of a " smoke." Ideally, the method used should reproduce as closely as possible the conditions under which sensitization takes place naturally in the factory. On this basis methods (1), (2) and (3) are not "natural," but were used in most of the work to be reported because, with (1) and (2) at least, good sensitization of a high proportion of the animals was achieved. Method (4) was used because lanoline is known to assist the absorption of fat-soluble substances, and because of the clinical observation that people with greasy skins seemed more liable to " tetryl " dermatitis. It was, however, unsuccessful. Method (5) produced a marked skin-reaction in only one of the eight animals used, but six of these animals were anaphylactically sensitive (detailed results in Table I and Fig. 1) ; these results may be of significance as regards normal conditions of sensitization of workers.

Albino or cream and white guinea-pigs, weighing 180–220 g. at the beginning of sensitization, were used throughout.

(1) *Intradermal injection.*—0·01 c.c. of M/100 solution of " tetryl " in propylene glycol was injected intradermally into the skin of the flank six times, in the course of a fortnight. After a further week the animals were tested by the application to the epilated skin of the belly of a throat swab dipped in the test solution, usually M/100 in acetone or dioxan. The area covered had a diameter of about 1 cm., and a dozen or more different solutions could be tried on the same animal without difficulty. Twenty-four hours later the animals were examined ; reactions were read as follows :

++ Very marked erythema, or oedema.
+ Marked erythema.
+— Slight definite erythema.
tr. Trace or doubtful reaction.

If necessary the area tested was cleaned with acetone to remove the stain of the test solution and facilitate reading. All test solutions were also painted on several control animals to ensure that they would not act as direct irritants.

This method was successful in sensitizing almost all of the animals used. A control group treated with injections of propylene glycol alone showed no sensitization under these conditions.

If animals sensitized by this method (or method (2)) were kept for re-testing, their sensitivity was maintained by a weekly or, better, a fortnightly injection of " tetryl " made up as in (1) (" maintenance dose "). They were not usually tested until at least 7 days after the last maintenance dose ; sensitivity seemed to be highest about the tenth day. Small immature animals were used, since they seemed to be more readily sensitized and to give stronger skin-reactions. Once the animals were

TABLE I.—*Skin and Anaphylactic Reactions of Animals Sensitized by Inhalations of Tetryl "Smoke."*

Guinea-pig number.		16.v.44. Skin sensitivity. "Tetryl."	T.N.P.*	Anaphylaxis tests.			
Females	1	tr.	tr.	+−		18.v.44	Dale *in vitro* technique.
	2	,,	,,	+		,,	
	3	,,	−	−		16.v.44	
	4	+−	+−	+		,,	
Males	5	−	−	+	1¼ mins. (mild symptoms)	18.v.44	Intravenous technique.
	6	tr.	tr.	+++	1¼ mins. (died 3 mins.)	16.v.44	
	7	,,	,,	−	(discomfort only)	18.v.44	
	8	,,	,,	+	1¾ mins. (mild symptoms)	16.v.44	

All the animals received 6 ½-hour exposures to a "smoke" of "tetryl" over the course of 14 days, starting 21.iv.44.

Technique.—The *in vitro* tests were done with the isolated uterus by the Dale technique; the +, etc., signs represent the intensity of the sensitivity as judged by the amount of antigen (picrylgelatin) needed to provoke the best reaction. None of these reactions were "maximal," i.e. gave contractions equal to those provoked by an optimal dose of histamine. The males were tested by injecting 0·1 c.c. of picrylgelatin into the vein of the thigh, which was exposed by a small cut made under local anaesthesia. Reactions were read as follows:

\+ Mild definite symptoms; several coughs, and laboured breathing.
++ Severe symptoms with convulsions and recovery.
+++ Severe symptoms with convulsions followed by death.

The *time of onset* of symptoms after the completion of the injection follows the + signs.

* In this and following Tables T.N.P. = trinitrophenetole.

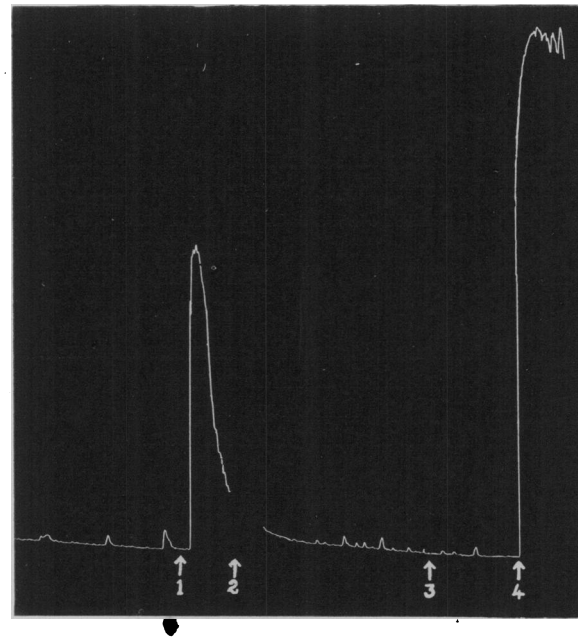


FIG. 1.—Uterine reaction (one horn) of guinea-pig sensitized by inhalation of "tetryl." 1. Antigen No. 28 (picrylgelatin); $1/10^3$ dilution. 2. Wash. 3. Antigen No. 28; $1/10^3$ dilution. 4. Histamine (sufficient to give maximal reaction).

sensitized, fortnightly maintenance doses were sufficient to preserve their sensitivity indefinitely, although the reactions on adult guinea-pigs were weaker, being seldom more than +.

(2) *Subcutaneous implantation of pellets.*—Pellets of " tetryl " weighing 10 or 25 mg. were made in a tablet-machine. Under light ether anaesthesia a cut was made through the skin of the shaved flank, and the capsule pushed with forceps laterally into the subcutaneous tissue until it could be palpated ½ in. to 1 in. from the incision. Collodion was then applied to the incision, which always healed rapidly. The capsule was removed on the fourteenth day by cutting over the point where it could be palpated and squeezing it out, the incision being again closed with collodion. As far as could be judged by the loss of weight of the pellet, absorption from both sizes amounted to 2–4 mg. After a further week the animal was tested as in (1). This method also sensitized nearly all animals, giving slightly more ++ reactions than method (1).

(3) *Scald method.*—The skin of the lower part of the flank was scalded by applying lightly for 1½ seconds the base of a cylindrical aluminium pot (diam. 1 in.) containing boiling water. M/10 " tetryl " in acetone was then applied on a swab to the scalded area, and this was repeated on the second and fourth days. At the beginning of the second week the scalding and applications were repeated on the other side ; the animals were tested as in (1) after a further week. In a small series sensitization was absent or doubtful, and the method was not pursued.

(4) *Inunction.*—A 1 per cent. suspension of " tetryl " in lanoline was made up, and approximately 0·1 g. of this was rubbed well into the epilated skin of the belly. Six treatments over the course of a fortnight were given, and the animals tested as in (1) after a further week. In the small group of animals used none showed any sensitization.

(5) *Inhalation of a " smoke."*—Eight animals were put in a chamber of 580 l. capacity (as described by Campbell, 1936) and exposed for 30 minutes to an artificial smoke of " tetryl " particles, made by blowing compressed air through a 10 per cent. solution of " tetryl " in acetone.* A rough colorimetric analysis of the air showed a concentration of about 0·4 mg. "tetryl " per litre ; the maximum particle size can be calculated as about 2–3μ. Six treatments were given ; the average ventilation of animals of the size used (250 g.) is about 6–8 l. per hour, and so each animal should have absorbed in all about 7–10 mg. Results are shown in Table I.

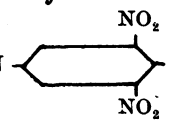
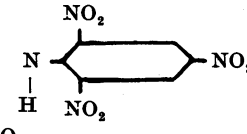
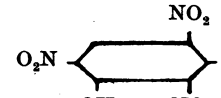
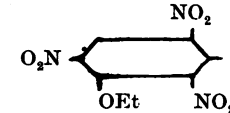
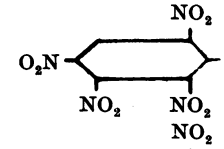
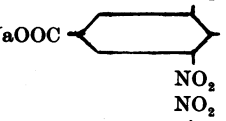
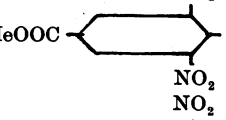
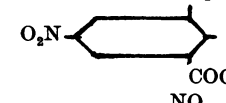
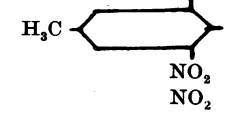
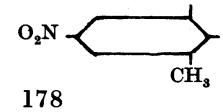
Cross-reactions.

A satisfactory method of sensitization to " tetryl " having been found, it became possible to study the nature of the group responsible. It is generally assumed that simple chemical substances which cause sensitization do so by virtue of their power to couple with proteins, thus producing antigens, after introduction into the body. In the case of " tetryl " it was at first thought probable that the sensitizing antigen would contain the reactive methylnitramino group ; this would imply coupling of " tetryl " with protein through one of its other functional groups. It is known that γ-trinitrotoluene condenses readily with amino-acids by elimination of a nitro group (Barger and Tutin, 1918), and a similar reaction with " tetryl " seemed not unlikely. If this were so, derivatives of " tetryl " in which one of the nitro groups was replaced by a different group, or in which one of the free positions of the benzene ring of " tetryl " was occupied by a substituent, should be able to elicit a skin reaction in " tetryl "-sensitized guinea-pigs. A number of such compounds were prepared and tested with completely negative results, except for a minor reaction with ethoxy-" tetryl " (Table II).

It was then discovered that the " tetryl "-sensitized guinea-pigs reacted strongly to picryl chloride and to 2 : 4 : 6-trinitrophenetole, i.e. to two compounds which

* Apparatus for this experiment was kindly prepared by Mr. J. E. Lovelock.

TABLE II.—*Results of Cross-reactions.*

"Tetryl"*		$-N(NO_2).CH_3$	+++
Picric acid* or its Na salt	,,	$-ONa$ or OH	−
Trinitrobenzene (symm.)	,,	$-H$	−
Trinitrotoluene (symm.)	,,	$-CH_3$	−
Trinitrobenzoic acid (symm.) Na salt	,,	$-COONa$	−
Trinitrobenzoic acid (ethyl ester)	,,	$-COOEt$	−
Picrylglycine* (neutralized c. NaOH)	,,	$-N(H)CH_2.COONa$	−
Picrylglycine (ethyl ester)	,,	$-N(H)CH_2.COOEt$	+
Trinitrophenetole*	,,	$-OEt$	+++
Picramide	,,	$-NH_2$	+
Methyl picramide	,,	$-N(CH_3)H$	++
Dimethyl picramide	,,	$-N(CH_3)_2$	+
Picryl chloride	,,	$-Cl$	++
Dipicrylamine	,,		−
Hydroxy-"tetryl"*			−
Ethoxy-"tetryl"*		,,	+−
Nitro-"tetryl"		,,	−
"4-acid"* (neutralized)		,,	−
"4-acid" (Me ester)		,,	−
"2-acid"* (neutralized)		,,	−
"4-Methyl compound"		,,	−
"2-Methyl compound"		,,	−

contained easily replaceable groups in the position corresponding to that of the methylnitramino group of " tetryl " itself. This directed attention to the alternative possibility that " tetryl " might couple with the amino groups of a protein by elimination of the methylnitramino group, the lability of which is indicated by the ease of hydrolysis of " tetryl " to give picric acid and by the reaction of " tetryl " with aniline to give 2 : 4 : 6-trinitrophenylaniline (van Romburgh, 1890). A renewed study of cross-reactions in the light of this idea showed that the power to elicit skin reactions in " tetryl "-sensitized guinea-pigs extended to picramide and its *N*-methyl and -dimethyl derivatives and to ethyl *N*-2 : 4 : 6-trinitrophenylaminoacetate ; it was lacking in *sym*-trinitrobenzene itself and in derivatives thereof substituted in the 1-position with groups which could not take part in coupling with proteins (Table II).

It thus appeared in general that compounds themselves containing the *N*-2 : 4 : 6-trinitrophenylamino group or potentially capable of forming such a grouping by combination with the amino groups of protein shared the property of eliciting the skin reaction, from which it could be deduced that this group was the essential determinant in the antigen formed by combination of " tetryl " with proteins in the manner already indicated.

It must be noted that two exceptions have been observed to the above general statement, namely, *N*-2 : 4 : 6-trinitrophenylaminoacetic acid (picrylglycine) and di-2 : 4 : 6-trinitrophenylamine (dipicrylamine) ; no final explanation of the anomalous behaviour of these two compounds can be offered, but it may be noted that they differ from the compounds which gave positive results chiefly in their pronounced acidic character. That the glycine derivative does conform in other respects to the theory is shown by immunological experiments to be described below.

Preparation of Picrylprotein Antigens.

The evidence concerning the nature of the hapten group involved in sensitization to " tetryl " which has been given in the previous section would clearly be strengthened if skin sensitization could be correlated with the formation of other antibodies specifically directed against this group, or if the process of sensitization could be influenced in any way by systemic immunization with an antigen containing such groups as determinants. For the study of both these possibilities it was necessary to prepare derivatives of protein analogous with those supposedly formed by combination with " tetryl," i.e. containing 2 : 4 : 6-trinitrophenyl residues as substituents in their free

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EXPLANATION OF TABLE II.

Solutions in acetone at M/100. Those marked * were also used in solution at M/10, without significantly different results. The + signs in this Table refer to the number of guinea-pigs showing reactions, as well as to their intensity.

Note.—The less familiar of the above compounds were prepared according to the following methods :

Picrylglycine, see K. Hirayama. Hoppe-Seyler's *Z.*, **59**, 290 (1909).

Picrylglycine ethyl ester. 2·35 g. glycine ester was dissolved in 5 c.c. alcohol and added to a solution of 2·5 g. picryl chloride in 30 c.c. alcohol. An oil separated at once which crystallized on scratching. Recrystallized from alcohol ; m.p. 92–93° C. Found (Dumas) : N. 17·7 per cent. $C_{10}H_{11}O_8N_4$ requires N, 17·9 per cent.

2 : 4 : 6-*Trinitro*-3-*ethoxy*-*methylnitramine.* See P. van Romburgh, *Rec.*, **8**, 274 (1889).

3 : 5-*Dinitro*-4-*methylnitraminobenzoic acid.* See F. Reverdin and A. de Luc, *Ber.*, **41**, 502 (1908).

3 : 5-*Dinitro*-4-*methylnitraminobenzoic acid, methyl ester.* See F. Reverdin and A. de Luc, *op. cit.*

3 : 5-*Dinitro*-2-*methylnitraminobenzoic acid* was prepared from 2-methyl-amino-3 : 5-dinitro benzoic acid under the conditions used for the preceding compounds (F. Reverdin and A. de Luc, *op. cit.*). The compound crystallized from chlorobenzene in hexagonal plates, m.p. 160° C.

3 : 5-*Dinitro*-4-*methylnitraminotoluene.* See P. van Romburgh, *Rec.*, **3**, 404 (1884).

3 : 5-*Dinitro*-2-*methylnitraminotoluene.* See P. van Romburgh, *Rec.*, **3**, 396 (1884).

amino groups. Such artificial antigens were conveniently prepared by the use, not of " tetryl " itself, but of the more reactive picryl chloride.*

Method 1 (Antigen No. 43).—65 c.c. of dialysed rabbit serum containing 2·5 g. of protein were diluted with an equal volume of dioxan and the minimum amount (10 c.c). of 0·5 *N* NaOH to keep the protein in solution. The solution was stirred in a bath at 40° C., and 0·9 g. of picryl chloride dissolved in 8 c.c. of dioxan was added slowly over 1½ hours. Small amounts of 0·5 *N* NaOH were added concurrently to keep the *p*H of the solution alkaline to cresol red. Stirring was continued for a further 20 mins. after the addition of picryl chloride was completed. The solution was dialysed against running tap-water for 15 hours, after which the volume was 210 c.c. The precipitate which had appeared was dispersed with 5 c.c. of 0·5 *N* NaOH and 115 c.c. alcohol added; the protein was then precipitated by the addition of 15 c.c. 2 *M* acetate buffer at *p*H 4·6, and was collected by filtration. It was resuspended in 100 c.c. water, dissolved with 5 c.c. 0·5 *N* NaOH, diluted with 200 c.c. alcohol and re-precipitated with 15 c.c. acetate buffer. This purification was repeated a second time. The product was re-suspended in water, dissolved with the minimum amount of NaOH, and dialysed against running tap-water for two days. A considerable amount of precipitate separated. This was filtered off and suspended in 100 c.c. water. A dry weight determination indicated 2·6 per cent. solid. Colorimetric determination using picryl-glycine as a standard showed that the product contained 3·8 per cent. picryl-groups estimated as picrylglycine. The concentration of protein was adjusted to 2 per cent. and the suspension was used for intramuscular or intraperitoneal injection. A dry weight determination on the filtrate (55 c.c.) gave 0·79 per cent. solid, and of the solid a content of 4·2 per cent. picryl estimated as picrylglycine. This solution was used for intravenous administration.

This general method was used for preparing the antigens for immunization of rabbits. The products were frequently largely insoluble, but nevertheless acted as satisfactory immunizing antigens.

Method of estimating picryl groups.—Standard solutions (made from a stock solution of picrylglycine containing 1 mg. per c.c. in glacial acetic acid) of 0·1, 0·2 and 0·5 mg. were made up to 4 c.c. with glacial acetic acid, 1 c.c. of concentrated HCl was added, and also an amount of plain serum with a protein content approximately equal to that of the solution to be tested. The solution of coupled protein to be tested (0·2 to 5 c.c.) was mixed with 4 c.c. glacial acetic acid and 1 c.c. HCl, and together with the standards was heated for 40 mins. in a boiling water-bath to hydrolyse.

After hydrolysis the tubes were cooled and zinc dust was added in two lots, the first of about 800 mg., and after 3 mins. a further 400 mg. The reduction took in all 5 mins. The solutions were then decanted from the zinc into 10 c.c. graduated cylinders and the zinc washed several times with water, the final volume being made up to 10 c.c. The solutions were filtered into 15 c.c. graduated cylinders and the volumes of filtrate adjusted to 8 c.c. 1 c.c. of 1 per cent. sodium nitrite solution was added and, after 3 mins., 1 c.c. of 5 per cent. sulphamic acid solution. After 2 mins. 2 c.c. of 1·0 per cent. naphthylethylenediamine hydrochloride was added, and the colours were compared after 10 mins.

Method 2.—This method was used for preparing picrylgelatin; some lots of picryl-serum-protein were also made by it (using a lower temperature). The product was always of good solubility, and was used for *in vitro* precipitin tests and for tests on anaphylaxis.

* *Note.*—Both tetryl and trinitrophenetole may be used in this reaction if the pH is kept at 8·5. The potassium salt of trinitrophenetole will also react readily with protein at this pH, and has the additional advantage of being water-soluble. Antigens prepared with any of these compounds give the same sort of reactions with antisera as those prepared with picryl chloride.

Preparation of antigen 28.—2 g. of gelatin (Gold Leaf) were dissolved in 40 c.c. of water at 70°; 0·6 g. of picryl chloride in 10 c.c. of alcohol (warmed to dissolve) was added in ½ c.c. quantities, *N* NaOH being added drop-wise from a pipette to keep the *p*H at about 8·0. The solution was then dialysed overnight, precipitated with a few c.c. of dilute acetic acid and redissolved by warming in 20 c.c. of water, with sufficient alkali to bring the substance into solution. The precipitation and re-solution were repeated twice; after the final re-solution the *p*H was adjusted to 7·4, and NaCl was added to bring the concentration of salt to 0·85 per cent. The concentration of protein was adjusted to 2 per cent. before use. Picryl-groups = 5·0 per cent. as picrylglycine.

Rabbit-antiserum.—In order to have a standard of comparison for the various preparations of antigen, an "anti-picryl-rabbit-serum" antiserum was made. Two rabbits were used; each received, during a seven weeks' course, 18 injections totalling approximately 300 mg. of "picryl-rabbit-serum" (prepared as in Method 1). This was given intravenously when the preparations were sufficiently soluble, otherwise intramuscularly or intraperitoneally. The animals were rested for a week; then a further two weeks' course was given, amounting to 60 mg.; 50 c.c. of blood were taken from the ear on the fifth and seventh days after the last injection. The fortnightly courses and bleedings were continued as long as serum was needed. The ring-test titres of the sera, against an antigen prepared by method 2 with horse-serum, varied between 1/125 and 1/625 of a 1 per cent. solution. The "equivalence point" of a bulked lot of serum was about 1/50 of a 1 per cent. solution. These sera, although not very strong, were quite satisfactory for the purpose.

RESULTS.

The occasional presence of circulating precipitins in sensitive animals has been recorded by Landsteiner and Chase (1937), and Cannon and Marshall (1940) claim to have demonstrated circulating antibody, by their collodion-particle technique, in a number of humans sensitive to egg-albumin or insulin. It has, however, been impossible

TABLE III.—*Effect of Parenteral Immunization of Sensitized Guinea-pigs.*

Date.	Test animals.				Controls.			
G.P. No.:	1.	2.	3.	4.	5.	6.	7.	
13.vii.44	250	280	260	320	300	310	350	Weights (g.).
13.vii.44 to 24.vii.44	All received * course of sensitization (·03 c.c. dose)							
1.viii.44	+−	+	+	++−	++	+	+	Sensitivity to "tetryl."
	+	tr.	+	++−	++−	+−	+−	Sensitivity to T.N.P.
2.viii.44 to 14.viii.44	6 intraperitoneal injections of Antigen 65† (0·25 c.c.)				*Nil*			Course of immunization of test animals.
7.viii.44	"Maintenance dose" of "tetryl" to all animals							
21.viii.44	++	++	+	++	tr.	−	−	Serum: precipitins.‡
	+	++	+	+	++	+	++	Sensitivity to "tetryl."
	+	+	+	++	++	+	+	Sensitivity to T.N.P.

* In this and subsequent Tables the course of sensitization was as follows: "Tetryl" was made up in propylene glycol in a M/100 (0·29 per cent.) solution; 0·01 to 0·03 (according to weight) of this was injected intradermally 3 times a week for 2 weeks.

† Antigen 65 = "picryl-guinea-pig-globulin": 2 per cent. solution.

‡ In this and subsequent Tables, estimated as follows: A few drops of blood were taken from the ear and the separated serum put up in ring test at various dilutions of antigen, usually 1/5 to 1/625 dilutions of a 1 per cent. antigen. + signs refer both to titre and intensity of reaction. A ++ reaction is one giving a very marked ring at 1/25, a definite sharp ring at 1/125, and sometimes a slight reaction at 1/625.

to demonstrate with certainty, by the ring test (using several dilutions of antigen), the presence of circulating precipitins in these " tetryl "-sensitized animals, although doubtful low-titre results were often given by the sera taken from animals a few days after the end of the initial course of sensitization. Possibly the degree of sensitivity of these animals was not high enough to allow of the appearance of precipitins in the serum ; but it seems more likely, from the results which follow, that demonstrable circulating precipitins are a " by-product," and are not directly relevant to the phenomenon of skin reactivity.

The results obtained using picrylproteins as antigens are detailed in Tables III–IX. Table III shows the effect of a course of intraperitoneal immunization with such antigens upon sensitive animals. It will be seen that the sensitivity is neither significantly increased nor depressed. Tables IV and V show a similar lack of effect of either previous or concurrent immunization on the sensitization process, and demonstrate (Table IV, *a*) the complete lack of correlation between the skin and the precipitin reactions. In several other groups of guinea-pigs parenterally immunized, there was similarly no evidence of skin sensitivity of the " contact " type.

From these results it might be suggested that our picryl antigens have no effect on " tetryl "-sensitized animals, and that the deduction that sensitivity to " tetryl " is dependent on the addition of picryl groups to body-proteins is incorrect. However, results on anaphylaxis, shown in Tables I and VI and Figs. 1–4, put this suggestion out of court. It was found that in almost every case intravenous injection of picryl-antigen (picrylgelatin* in the case of the results shown in Table VI) into sensitized guinea-pigs will produce typical anaphylactic symptoms, frequently leading to death. By the Dale technique, the uteri of sensitive animals give contractions, followed by desensitization, after contact with high dilutions of antigen (Fig. 2, *a*) ; normal uteri are unaffected, even by much larger quantities. Landsteiner and Chase (1937) had similar results with animals sensitized to various simple chemicals, including picryl chloride, and injected intravenously with the appropriate antigenic conjugate, and Fierz, Jadassohn and Stoll (1937) report anaphylaxis in animals previously injected with a diazonium complex.

We may here refer to Table I and Fig. 1, showing the results on guinea-pigs sensitized by inhalation, although they constitute something of a side-issue. These results are of considerable interest from the point of view of the industrial sensitization of humans. Although the skin reactions were not striking, tests for anaphylaxis were definitely positive in 6 out of 8 animals and, since simple skin application of " tetryl " was found to be ineffective in producing sensitivity in an earlier group of guinea-pigs, it is probable that the path of sensitization is *via* the lung ; this gives an additional emphasis to the need for reducing the dust-hazard in factories where chemicals able to sensitize are handled. It is likely that alteration in technique might have produced more striking skin reactions.

Figs. 2, *a* and *b*, show respectively the uterine and intestinal *in vitro* reactions of a sensitized guinea-pig. Fig. 3 (upper tracing) shows the reaction of the uterus of a guinea-pig injected with 0·5 c.c. of the serum of a sensitive guinea-pig ; it should be noted that this serum contained no precipitins demonstrable by the ring-test. Fig. 4 shows a similar passive sensitization effect, this time by the use of the serum of a guinea-pig immunized intraperitoneally with picrylprotein, and not skin sensitive. It may be that the Dale test is very much more sensitive in detecting circulating precipitin-like antibodies than the ring-test ; or possibly that a type of antibody present in sensitive guinea-pigs but not demonstrable as a precipitin is able to sensitize

* This antigen was used as being less likely to produce aberrant results owing to the protein " carrier." Other picrylproteins, including picryl-guinea-pig globulin, are equally effective.

TABLE IV.—*Showing* (a) *Lack of Correlation Between Circulating Precipitins and Sensitivity* (*Cages I and II*); (b) *Failure of Previous Immunization to Affect Sensitization* (*Cages I and III*).

Date.	Cage No. I.				II.				III.							
G.P. No.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	
19.vi.44	205	180	230	190	180	180	185	190	..							Weights (g.).
19.vi.44 to 30.vi.44	6 intraperitoneal doses of Antigen 65*				Course of sensitization				*Nil*							
12.vii.44	−	−	−	−	++	++	++−	++−	..							Skin sensitivity to "tetryl."
,,	−	−	−	−	++	++	++−	++−	..							Skin sensitivity to T.N.P.
13.vii.44	++	++	++−	+	−	−	−	−	..							Serum: precipitins.
,,	300	300	330	300	..				250	300	300	280	320	260	310	Weights (g.).
13.vii.44 to 24.vii.44	Course of sensitization				..				Course of sensitization							..
31.vii.44	+	+−	+	+	..				+−	+	++	+	++−	+	+	Skin sensitivity to "tetryl."
,,	+−	Tr.	+	+−	..				+	+−	++−	tr.	++−	+	+−	Skin sensitivity to T.N.P.
,,	390	390	440	400	..				375	440	370	400	430	335	420	Weights (g.).

* Antigen 65 = "picryl-guinea-pig-globulin": 2 per cent. solution.

TABLE V.—*Effect of Concurrent Immunization on Sensitizability.*

Date.	Test animals.					Control animals.					
G.P. No.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	
19.x.43 to 5.xi.43	9 subcutaneous injections of Antigen 27*					*Nil*					..
27.x.43 to 5.xi.43	Course of sensitization for all animals.										
25.x.43	200	255	200	210	210	185	230	205	225	190	Weights (g.).
1.xi.43	+	++	++	+−	++	−	−	−	−	−	Serum: precipitins.
11.xi.43	++	++	++	++	++	?†	−	?†	−	?†	,, ,,
,,	−	+--	tr.	+	−	++	tr.	+−	+	+−	Sensitivity to "tetryl."
,,	+−	+	,,	+	+	++	+	+	+	+	,, T.N.P.

* Antigen 27 = "picryl-rabbit-serum": 2 per cent. solution.

† Doubtful reactions at higher concentration of antigen, such as are sometimes observed immediately after the course of sensitization.

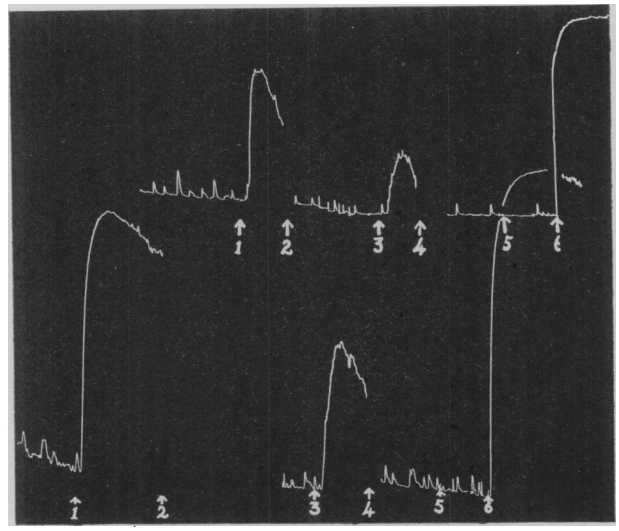

FIG. 2*a*.—Uterine reaction (both horns) of guinea-pig sensitized by intradermal injections of "tetryl." Upper tracing: 1. Antigen No. 28; $1/10^6$ dilution. 2. Wash. 3. Antigen No. 28; $1/10^5$ dilution. 4. Wash. 5. Antigen No. 28; $1/10^5$ dilution. 6. Histamine.

Lower tracing: 1. Antigen No. 28; $1/10^5$ dilution. 2. Wash. 3. Antigen No. 28; $1/10^5$ dilution. 4. Wash. 5. Antigen No. 28; $1/10^5$ dilution. 6. Histamine.

This record shows the difficulty of desensitizing the uterus of a sensitized animal; full desensitization is achieved at 5, in both tracings.

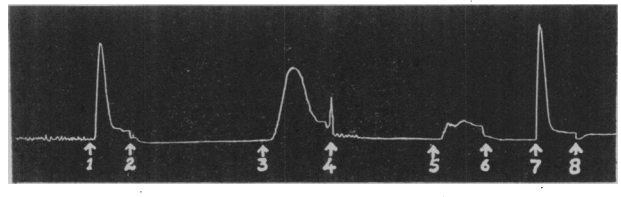

FIG. 2*b*.—Intestinal reaction of guinea-pig sensitized by intradermal injections of "tetryl." 1. Antigen No. 28; $1/10^5$. 2. Wash. 3. Antigen No. 28; $1/10^4$. 4. Wash. 5. Antigen No. 28; $1/10^3$. 6. Wash. 7. Histamine. 8. Wash.

This record shows the difficulty of desensitizing the intestine of a sensitized animal; desensitization is not complete, though increasing amounts of antigen give decreasing reactions. Normal intestine does not react to this antigen even at $1/10^2$ dilution.

uterine tissue, as well as is the ordinary precipitin. Quantitative work on these two methods of passive sensitization is in progress.

Fig. 3 (lower tracing) shows also that picrylglycine, which acts as a powerful inhibitor of the rabbit "anti-picryl" serum, is equally an inhibitor of the uterine reaction. The contraction of the uterus of a strongly sensitized guinea-pig is usually only partially inhibited; the reaction shown is from a normal guinea-pig sensitized rather weakly by intracardiac injection of 1 c.c. of the serum of a sensitive guinea-pig.

Table VI shows, in group (*a*), the failure of large quantities of antigen to elicit any symptoms in normal guinea-pigs, and in group (*b*) the reactions elicited in whole guinea-pigs sensitized to "tetryl." Groups (*c*), (*d*) and (*e*) show results of attempts to inhibit the reaction by various means. It will be seen that desensitization, even with protein-antigen (group (*d*)), is difficult, although one, or better two, subcutaneous

injections previous to the shocking dose exert a definite protective effect. Picrylglycine injected the day before the shocking dose (group (*e*)) has no effect, presumably owing to its rapid excretion, while if it is injected together with the shocking dose (group (*c*)), it seems to exert, as would be expected from the *in vitro* results, a slight protective effect. The number of animals used is, however, rather small.

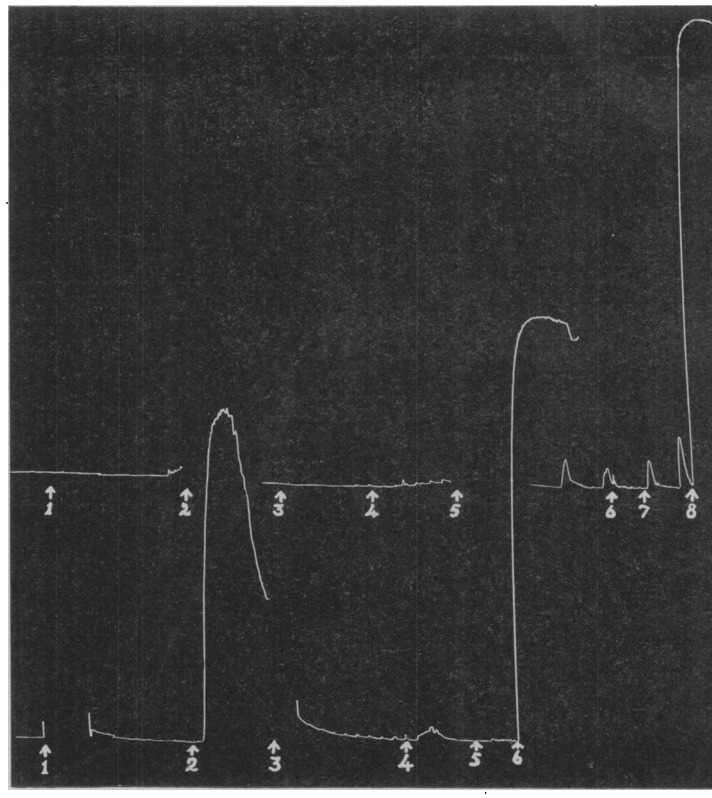

FIG. 3.—Uterine reaction (both horns) of guinea-pig sensitized passively by injection intracardially of 1 c.c. of serum from a "tetryl"-sensitized guinea-pig (showing no precipitins by ring test); also showing inhibition of reaction by "picrylglycine."

Upper tracing: 1. 0·5 c.c. picrylglycine M/20 in saline at *p*H 7·4. 2. Wash. 3. 0·5 c.c. picrylglycine M/20 in saline at *p*H 7·4. 4. Antigen No. 28, $1/10^3$. 5. Wash. 6. Antigen No. 28, $1/10^3$. 7. 0·5 c.c. picrylglycine M/20 in saline at *p*H 7·4. 8. Histamine.

Lower tracing: 1. Wash. 2. Antigen No. 28, $1/10^3$. 3. Wash. 4. Antigen No. 28, $1/10^3$. 5. 0·5 c.c. picrylglycine M/20 in saline at *p*H 7·4. 6. Histamine.

This record shows, in the lower tracing, a good but not maximal reaction to a fairly large dose of antigen, followed by desensitization; picrylglycine is added at 5, and histamine at 6, to demonstrate that picrylglycine does not interfere with normal contractility. The upper tracing shows complete inhibition of contraction in the presence of picrylglycine, followed by desensitization.

Table VII shows the effect on sensitive guinea-pigs of circulating antigen introduced intraperitoneally. There is a transient diminution, but not a suppression, of the skin reaction. Landsteiner and Chase (1937) record similar results. Tables VIII and IX show an interesting contrast between two types of "hapten." Picrylglycine, which is chemically inactive, has no effect whatever on the skin reaction, while trinitrophenetole, which can combine with protein to form a full antigen, has a

TABLE VI.—*Anaphylactic Reactions of Sensitive Animals.*

	Date.	G.P. No.	Sensitivity.	Antigen.	Dose.	Route.	Time (mins.).	Result.
(*a*)	21.2	1	—	45	0·5	I–V	*Nil*	—
Normals, 4;	,,			,,	,,	I–C	,,	—
negative	,,	2	—	,,	,,	I–V	,,	—
controls.	,,	3	—	,,	,,	,,	,,	—
	,,			,,	,,	I–C	,,	—
	,,	4	—	,,	,,	I–V	,,	—
(*b*)	,,	5	+—	,,	0·1	,,	½–4½	D
Sensitive	,,	6	+—	,,	,,	,,	½–3	D
guinea-pigs, 9;	,,	7	+	,,	,,	,,	½–3	D
positive	,,	8	+	,,	,,	,,	3–R	+
controls.	,,	9	+	,,	0·15	I–C	1–5½	D
	,,	10	+—	,,	0·1	I–V	? slight	+— —
	,,	11	+	,,	,,	,,	½–3½	D
	,,	12	+—	,,	,,	,,	½–1	D
	,,	13	+—	,,	,,	,,	½–2	D
(*c*)	,,	14	+	45 : 1 pt. PG : 2 pt.	0·3	,,	1½–R	+
Sensitive	,,							
guinea-pigs, 6.	,,	15	+	45 : 2 pt. PG : 2 pt.	0·4	,,	1½–7	D
Mixture of								
antigen and	,,	16	+—	45 : 1 pt. PG : 2 pt.	0·3	,,	*Nil*	—
picryl-								
glycine M/20.	,,	17	+	,,	,,	,,	1½–4	D
	,,	18	+	,,	,,	,,	1½–4½	D
	,,	19	+—	,,	,,	,,	1½–R	++
(*d*)	,,	20	+	45	0·2	S–C		
Sensitive	22.2			,,	0·5	I–P		
guinea-pigs, 10.	23.2			,,	0·1	I–V	3½–R	+ (delayed)
1 or more								
previous	21.2	21	+	,,	0·2	S–C		
injections	22·2			,,	0·1	I–V	½–3	D
of antigen	21.2	22	+—	,,	0·2	S–C		
(I–P or I–M).	22·2			,,	0·1	I–V	*Nil*	—
	22·2	23	+	,,	0·5	S–C		
	23·2			,,	0·15	I–V	¼–3	D
	22.2	24	+—	,,	0·5	S–C		
	23·2			,,	0·1	I–V	? slight	+—
	22.2	25	+	,,	0·5	S–C		
	23·2			,,	0·1	I–V	¾–3½	D
	22·2	26	+	,,	0·5	S–C		
	23·2			,,	,,	I–M		
	24.2			,,	0·1	I–V	1½–R	++
	23·2	27	+	,,	0·5	I–M		
	24.2			,,	,,	I–P		
	25.2			,,	0·1	I–V	4½–R	+
	23.2	28	+	,,	0·5	S–C		
	24.2			,,	,,	I–P		
	25.2			,,	0·1	I–V	2–R	+
	22.2	29	+	,,	0·5	I–P		
	23.2			,,	,,	I–M		
	24.2			,,	0·1	I–V	1½–R	++
(*e*)	21.2	30	+	PG	0·5	I–C		
Sensitive	22.2			45	0·1	I–V	1–4½	D
guinea-pigs, 5.	21.2	31	+	PG	0·5	I–C		
1 previous	22.2			45	0·1	I–V	1–2	D
injection	21.2	32	+—	PG	0·5	I–C		
of picryl-	22.2			45	0·1	I–V	1½–3½	D
glycine M/20.	22.2	33	+—	PG	0·5	I–P		
	23.2			45	0·1	I–V	1½–R	++
	22.2	34	+	PG	0·5	I–P		
	23.2			45	0·1	I–V	½–2	D

Analysis of Results.

Group.	Total in Group.	Reaction. Death.	Severe.	Slight or *nil*.	Percentage surviving.	Percentage slightly or not affected.
(*a*) Negative controls	4	0	0	4	100	100
(*b*) Positive controls	9	7	1	1	22	11
(*c*) Mixture of antigen and PG	6	3	1	2	50	33
(*d*) Previous injection of antigen	10	3	3	4	70	40
(*e*) Previous injection of PG	5	4	1	0	20	0

Symbols, etc.

Antigen 45 = "picryl-horse-serum," 2 per cent. solution. PG = picrylglycine, M/20 solution, in saline, buffered at *p*H 7·4. I–V = Intravenous. S–C = Subcutaneous. I–M = Intramuscular. I–C = Intracardiac. I–P = Intraperitoneal. R = Recovery. D = Death.

Col. 3 shows grade of sensitivity, as tested on 20.ii.44.

„ 7 shows time of onset of symptoms and time of death, or recovery.

„ 8 shows intensity of reaction: D = death; ++ = severe, with convulsions; + = definite reaction, coughing and straining.

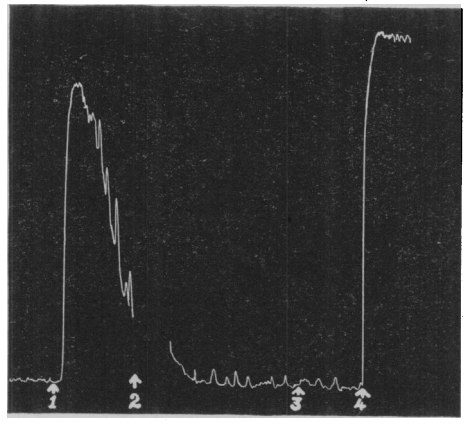

FIG. 4.—Uterine reaction (one horn) of guinea-pig sensitized passively by injection intracardially of 1 c.c. of the serum from a guinea-pig immunized by intraperitoneal injections of picryl-protein (showing precipitins but no skin-sensitivity).

1. Antigen No. 28, $1/10^4$. 2. Wash. 3. Antigen No. 28, $1/10^4$. 4. Histamine.

Note.—The capacity of the bath used for each horn was 15 c.c. The uteri were suspended in Ringer-Locke solution, the intestine (Fig. 2*b*) in Ringer-Tyrode solution and they were washed with the appropriate solutions. All solutions were kept at 38°C. 0·1 c.c. antigen was added at the dilution indicated.

slight but definite suppressive effect, rather more marked after 72 hours than after 24. It is suggested that a slowly formed "conjugate," rather than the simple hapten, is responsible for this partial suppression, which is thus analogous to the suppression by externally formed protein antigen shown in Table VII.

Finally, Table X shows the result of an attempt to confirm directly the hypothesis that sensitization occurs *via* the production of an antigenic conjugate with tissue protein. The first pair of experiments (Cage 1, test animals, and Cage 2, controls) gave an apparently positive result, but there was a possibility that the antigen used might contain unconjugated picryl chloride, which has an appreciable solubility in water. Therefore a similar antigen (picryl-guinea-pig-globulin) was treated with glycine at an alkaline *p*H in order to remove residual picryl chloride, a precaution taken by Landsteiner and Chase (1941) when demonstrating the possibility of producing

TABLE VII.—*Effect of Circulating Antigen : Temporary Reduction of Sensitivity Reaction.*

Date.	G.P. No.	Previously sensitized animals. Test animals. 1.	2.	3.	4.	Controls. 6.	7.	
23.viii.43	.	+−	−	+	tr.	+−	tr.	. Sensitivity to "tetryl."
		+	tr.	++	+−	+−	+−	. „ T.N.P.
17.ix.43	.	tr.	−	+−	tr.	+	tr.	. „ "tetryl."
		+−	+	++	+−	+	+	. „ T.N.P.
20.ix.43	.	Intraperitoneal dose (0·5 c.c.) of Antigen 25*				*Nil*		
21.ix.43	.	−	tr.	−	−	+	tr.	. „ to "tetryl."
		−	−	+−	−	tr.	+−	. „ T.N.P.
		"Maintenance" injection of "tetryl" to all animals.						
24.ix.43	.	tr.	tr.	tr.	tr.	+	+−	. „ "tetryl."
		„	+−	„	„	+−	+	. „ T.N.P.
28.ix.43 to 5.x.43	.	"Maintenance" injections of "tetryl" to all animals.						
5.x.43	.	−	−	−	−	−	−	. Serum : precipitins.
11.x.43	.	−	−	−	−	−	−	. „ „
		+−	+	+−	tr.	+	+	. Sensitivity to "tetryl."
		+	+−	+	+−	+	+	. „ T.N.P.

* Antigen 25 = "picryl-rabbit-serum" : 2 per cent. solution.

skin sensitization by the intraperitoneal injection of conjugates. A picryl-horse-serum antigen, similarly treated, was also used, to find whether a homologous protein was necessary. It will be seen that Cages 3 and 4 showed poor or negative sensitization, in spite of an increased dosage. This contrast strongly suggests that such sensitization as occurred was produced by the presumably minute amount of picryl chloride still present, and not by the conjugate itself.

The discussion which follows is confined to examples of skin sensitivity of the contact type.

DISCUSSION.

The recent work of the late Karl Landsteiner and co-workers and others has demonstrated without reasonable doubt that skin-sensitivity is an immunological reaction, i.e. that it is the result of the production of specific antibodies. The most likely way for this to happen from both chemical and biological evidence is *via* the production by the excitant of a "conjugate" with the body-proteins of the recipient animal. Such of the above-described results as parallel Landsteiner's support these conclusions.

This work has been directed towards the discrimination between the various types of antibody, namely, of those responsible respectively for the skin reaction, the anaphylactic reaction, and the "precipitin" reaction. In particular it is evident that there is no correlation between demonstrable circulating precipitins and skin reactivity (Tables III–V). Landsteiner and Chase (1940) show that typical skin sensitivity can be produced by the intraperitoneal injection of the excitant together with tubercle bacilli, i.e. that it is not essential that the skin should be the site of fixation of the antigen for skin-sensitivity to occur. In a short but suggestive communication (1942) they claim to have transferred the skin reaction passively by injecting into normal animals the washed centrifuged deposits from the peritoneal exudates of animals sensitized in this way, and suggest that the characteristic cells of such exudates, presumably mainly large mononuclears, carry a special sort of antibody.

TABLE VIII.—*Effect on Sensitivity of Circulating Hapten (Picrylglycine).*

Date.	G.P. No.	Previously sensitized animals. Test animals. 1.	2.	3.	4.	5.	6.	7.	Controls. 8.	9.	10.	11.	12.	13.	14.	
23.ix.43	.	+	+	+−	tr.	tr.	+−	+−	+	+−	+	tr.	+−	+−	+	Sensitivity to "tetryl."
		+	+−	+−	+−	tr.	+−	+−	+−	+−	+	tr.	+−	+−	+	,, T.N.P.
26.ix.43	.	Intraperitoneal injection (3·5 mg.) of picrylglycine*										*Nil*				..
27.ix.43	.	+	+	+	+−	+	tr.	+	+	+	+	+	+−	tr.	+	,, "tetryl."
		tr.	+−	+	+−	+−	tr.	+	+−	+−	+	+	+−	+−	+	,, T.N.P.
28.ix.43	.	"Maintenance injection" of "tetryl" to all animals.														
30.ix.43	.	+	+	+−	−	+	tr.	+−	+	+−	+−	+−	−	+	+−	,, "tetryl."
		tr.	tr.	tr.	−	+	+−	−	+−	tr.	+	tr.	−	+−	+	,, T.N.P.

* M/20 solution buffered at *p*H 7·4.

TABLE IX.—*Effect of Circulating Hapten (Trinitrophenetole).*

Date.	G.P. No.	Previously sensitized animals. Controls. 1.	2.	3.	4.	Skin-test 1 day after injection. 5.	6.	7.	8.	9.	10.	11.	Skin-test 3 days after injection. 12.	13.	14.	
27.ix.43	.	+	+	+	+−	+−	+	+−	+	+	tr.	+	+	+−	tr.	Sensitivity to "tetryl."
		tr.	+−	+	+−	+−	+	+−	+	+−	tr.	+	tr.	+	−	,, T.N.P.
5.x.43	.	"Maintenance" injection of "tetryl" to all animals.														
10.x.43	.	*Nil*				Intraperitoneal injection of 2·5 mg. T.N.P. in prop. glycol							*Nil*			..
11.x.43	.	+	+	+	+−	−	tr.	−	tr.	tr.	+−	+	+−	+	+	Sensitivity to "tetryl."
		+	+	tr.	+−	−	−	−	++	−	−	+	tr.	+	+	,, T.N.P.
12.x.43	.	*Nil*							*Nil*				I-P injection of 2·5 mg. T.N.P. in prop. glycol.			..
15.x.43	.	+−	+	+−	tr.	−	+−	tr.	+	tr.	+−	+	tr.	−	−	,, "tetryl."
		+−	+	+−	tr.	−	tr.	tr.	+	tr.	+−	+	−	−	−	,, T.N.P.

TABLE X.—*Attempts to Sensitize Animals by Intradermal Injection of Protein Conjugates.*

Date.	Cage No. I.					II.				Date.	III.					IV.				
G.P. No.	1.	2.	3.	4.		5.	6.	7.	8.		9.	10.	11.	12.		13.	14.	15.	16.	
19.vi.44	175	210	210	210	.	180	180	185	190	. 19.vi .	185	170	155	170	.	200	190	200	170	. Weights (g.).
19.vi.44 to 30.vi.44	6 intradermal doses of antigen				.	Course of sensitization				. 19.vii . to 1.viii	13 intradermal doses of antigen 65				.	13 intradermal doses of antigen 36*b*				. ..
12.vii.44 .	+	−	+	+−	.	−	−	−	−	. 9.viii .	++−	+	++	−+	.	+	+−	+	+−	. Serum : precipitins.
	+	−	+	+−	.	++	++	++−	++−	. .. .	+−	−	−	−	.	tr.	−	−	tr.	. Sensitivity to "tetryl."
	−	+	+	+−	.	+++	++	++−	++−	.	tr.	−	−	−	.	+−	−	−	−	. Sensitivity to T.N.P.
										14.viii .	++	−	++	++	.	−	−	−	−	. Serum : precipitins.
											tr.	tr.	−	+	.	tr.	−	tr.	tr.	. Sensitivity to "tetryl."
											tr.	−	−	+−	.	tr.	−	tr.	−	. Sensitivity to T.N.P.

Doses.—Cage No. I : 0·5 mg./dose of "picryl-guinea-pig-globulin" (untreated). (Antigen 64.)
„ II : (Controls) Standard course of sensitization with "tetryl" in propylene glycol.
„ III : 0·5 mg./dose of "picryl-guinea-pig-globulin" (Antigen 65), treated twice for 2 hours at 37° C. with 5 per cent. glycine at pH 8·5 and dialysed.
„ IV : 0·5 mg./dose of "picryl-horse-serum" (Antigen 36*b*), treated once for 2 hours at 37° C. with 5 per cent. glycine at pH 8·5 and dialysed.

This hypothesis seems the simplest that will explain all the facts. That a particular sort of antigen, a " skin-protein-conjugate," is not essential, is shown by the demonstration, by Landsteiner and Chase (1941), of skin sensitization resulting from intraperitoneal injection of " picryl-stromata," and indeed by all their experiments where the peritoneum and not the skin is the contact tissue; as also by the experiment detailed here in Table I, of sensitization occurring, almost certainly from absorption *via* the lungs. These experiments also suggest that, just as local production of antigen in the skin is not necessary, neither is local production of antibody.

Light is perhaps thrown on the question by the negative experiment shown in Table X. If the deduction is correct, that picryl-guinea-pig-globulin as such has been ineffective here in producing sensitivity, this experiment at first sight seems to disprove, by direct demonstration, the hypothesis of conjugation between the excitant and tissue protein. It also contrasts with the successful production of sensitivity, by Landsteiner and Chase (1941) by the intraperitoneal injection of full antigen (" picryl-stromata "), treated to ensure the absence of free picryl chloride. These workers do not record any attempt to produce skin sensitivity by *intradermal* injection of picryl-stromata; in the absence of such an experiment, one is nevertheless forced to the conclusion that the protein " carrier " plays an essential part. The production of skin sensitivity by intraperitoneal injection of picryl stromata, while it is not produced by injection of soluble picrylproteins, either intradermally or intraperitoneally, is evidently due to the special nature of the carrier. Such a particulate antigen, in which the protein, though homologous, is probably somewhat denatured during the preparation, is very different from antigens like those used in the experiment shown in Table X, which were freely soluble and showed no signs of denaturation. This suggests that an antigen acting like a " foreign body " may be an effective sensitizer, where a freely soluble antigen (though still able to produce precipitins) is not; and one is led back to consider the activities of the wandering cells. In general, indeed, substances such as dead tubercle bacilli, which produce a " foreign body " type of reaction, one, that is, associated with the large mononuclear cells, seem to favour the production of skin-sensitizing antibodies. Possibly the excitant, " tetryl," for example, or picryl chloride, conjugates with and kills the cells in its immediate vicinity (which are then taken up by mononuclears), and these dead cells act as antigens in the same way as the " picryl-stromata " of Landsteiner and Chase; it may be this reaction, rather than a conjugation of the excitant with proteins dissolved in the body fluids, that is essential for the production of sensitivity.

The hypothesis of a special sort of antibody, which may be carried in cells (though on occasion it may be free in the blood-stream), is unsatisfactory for reasons of economy, but seems to be needed to explain the lack of correlation between circulating antibodies, demonstrable as precipitins and able to produce passive anaphylaxis, and those presumed to be responsible for the skin reaction (but which may also be able to produce passive anaphylaxis when free in the blood-stream). There is a peculiar contrast between the evidently " systemic " nature of the sensitization process, occurring *via* the lymph- and blood-streams, and the localization to tissues of the characteristic " sensitivity " reaction. That this contrast is not merely a concentration effect, depending on a greater delicacy in the skin test, is shown by the absence of skin-reactions in guinea-pigs with plenty of circulating antibodies (Table IV). Some further factor or process must be postulated whereby antibodies are localized in the skin; and the hypothesis of such antibodies as being produced, and usually fixed, in cells, but in " wandering cells," would help to clear up the difficulties.

SUMMARY.

(1) The problem of sensitivity to "tetryl" (trinitrophenylmethylnitramine) has been investigated. Various methods of sensitization of guinea-pigs are described, and the skin reactions of successfully sensitized pigs to "tetryl" and allied compounds recorded. From these results, it is concluded that tetryl sensitizes by reacting with the recipient's body protein to form an antigenic "picrylprotein."

(2) An experiment is described whereby animals are sensitized by the inhalation of an artificial "smoke" of "tetryl."

(3) Methods of making picrylproteins *in vitro* are given, and by means of these the relation between circulating precipitins and the antibodies responsible for the skin reaction is investigated. It is shown that there is no correlation between these two types of immunological reaction.

(4) Sensitized animals are shown to exhibit anaphylactic phenomena on intravenous injection of picrylproteins, both *in vivo* and *in vitro* by the Dale technique.

(5) The effect of circulating antigen and hapten on skin sensitivity is investigated.

(6) An experiment is described whereby guinea-pigs are shown to be only weakly or not at all sensitized by the injection intradermally of picrylproteins made with either guinea-pig globulin or horse serum.

I am much indebted to Dr. C. R. Harington for advice and encouragement, and to Mrs. R. V. Pitt-Rivers for preparing the chemical compounds used in this work. Dr. A. J. Amor, of the Ministry of Supply, was kind enough to arrange for me to visit Royal Ordnance Factories in order to investigate and discuss conditions of industrial sensitization; and Mr. A. R. V. Steele, of the Ministry of Supply, gave much assistance on chemical problems. I wish also to express my gratitude to Dr. Jacques and Mr. Knapman, Superintendents of Royal Ordnance Factories, for giving me all facilities on my visits; and particularly to Dr. A. D. K. Peters, Senior Medical Officer at a Northern R.O.F., and her staff, for great help and friendly encouragment on my visits there.

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